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Depression of primary afferent-evoked responses by GR71251 in the isolated spinal cord of the neonatal rat

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1 The pharmacological profile of GR71251, a new tachykinin receptor antagonist, and its effect on the responses evoked by stimulation of primary afferent fibres were studied in isolated spinal cord preparations of neonatal rats. Potential changes were recorded extracellularly from a lumbar ventral root (L3-L5).

2 Bath-application of substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) at 0.01–3 μM to the spinal cord induced depolarization of the ventral root in normal artificial cerebrospinal fluid (CSF). The NK_1 agonist, acetyl-Arg^6-septide, and the NK_3 agonist, senktide, at 0.01–3 μM, also had potent depolarizing actions whereas two NK_2 agonists, β-Ala^{8}NKA$_{4-10}$ and Nle^{10}NKA$_{4-10}$, showed little depolarizing effects at 1 μM.

3 GR71251 (0.3–3 μM) caused a rightward shift of the concentration-response curves for SP, acetyl-Arg^6-septide and NKA with pA_2 values of 6.14, 6.75 or 6.70, respectively. The effects of GR71251 were readily reversible within 15–30 min after its removal. By contrast, GR71251 (1–5 μM) had little effect on the depolarizing responses to NKB and senktide.

4 GR71251 (1–3 μM) did not depress the depolarizing responses to bombesin, neuromedin B and gastrin-releasing peptide in normal artificial CSF.

5 Application of capsaicin to the spinal cord induced a depolarizing response, which was partially depressed by GR71251 (3–10 μM).

6 In the isolated spinal cord preparation, intense electrical stimulation of a dorsal root evoked a slow depolarizing response of the contralateral ventral root of the same segment. A similar slow ventral root depolarization was evoked by electrical stimulation of the ipsilateral saphenous nerve in an isolated spinal cord-saphenous nerve preparation. GR71251 (0.3–10 μM) dose-dependently depressed these slow ventral root potentials.

7 In the spinal cord-peripheral nerve preparation, conditioning stimulation of the saphenous nerve evoked an inhibition of the muscle nerve-evoked monosynaptic reflex lasting about 20 s. The late part of the inhibition was markedly depressed by GR71251 (1–3 μM).

8 The present results indicate that GR71251 is a potent and specific antagonist for tachykinin receptors in the spinal cord. The present study further provides evidence for the involvement of SP and NKA in the slow ventral root depolarization and the prolonged inhibition of monosynaptic reflex that are evoked by primary afferent stimulation.

Keywords: Tachykinin receptor; tachykinin antagonist; GR71251; substance P; bombesin

Introduction

We have previously reported that activation of primary afferent fibres induces two types of prolonged response in the spinal cord of the neonatal rat: a depolarization of the ventral root elicited by electrical or chemical stimulation of primary afferents (Yanagisawa *et al.*, 1982; Akagi *et al.*, 1985; Otsuka & Yanagisawa, 1988; Nussbaumer *et al.*, 1989), and an inhibition of the muscle nerve-evoked monosynaptic reflex elicited by electrical conditioning stimulation of the saphenous nerve (Yoshioka *et al.*, 1990). Both the slow ventral root depolarization and the prolonged inhibition of the monosynaptic reflex were markedly depressed by tachykinin antagonists, [D-Arg^1, D-Pro^2,D-Trp^{7,9},Leu^{11}]SP and/or spantide (Yanagisawa *et al.*, 1982; Akagi *et al.*, 1985; Otsuka & Yanagisawa, 1988; Nussbaumer *et al.*, 1989; Yoshioka *et al.*, 1990), which suggests the involvement of tachykinins in these responses. To clarify the functional roles of tachykinins and their receptors, however, further improvement of tachykinin antagonists in terms of potency and selectivity is needed. For example, some tachykinin antagonists were shown to have local anaesthetic actions at high concentrations (Post *et al.*, 1985; Yoshizawa *et al.*, 1987), and to act as antagonists on bombesin receptors (Folkers *et al.*, 1984; Yachnis *et al.*, 1984; Jensen *et al.*, 1984; 1988; Mizrahi *et al.*, 1985; Otsuka & Yanagisawa, 1988; Jensen & Coy, 1991).

Ward *et al.* (1990) recently developed a new tachykinin receptor antagonist, GR71251, which was shown to be selective for NK_1 receptor (Hagan *et al.*, 1990; Ward *et al.*, 1990; Hall & Morton, 1991; Ireland *et al.*, 1991). Furthermore, GR71251 did not depress the action of bombesin in the guinea-pig gallbladder (Ward *et al.*, 1990). In the present study we therefore examined the pharmacological profile of GR71251 in the neonatal rat spinal cord and its effects on the responses evoked by activation of primary afferent fibres. Preliminary accounts of this study have been presented elsewhere (Guo *et al.*, 1992; 1993).

Methods

Preparations

Isolated spinal cord preparations and spinal cord-peripheral nerve preparations from neonatal Wistar rats (Nihon Rat Co., Japan) aged 1 to 3 days of either sex were used (Akagi *et al.*, 1985; Nussbaumer *et al.*, 1989; Yoshioka *et al.*, 1990). Spinal cords below thoracic segments were used in the

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experiments in which the effects of GR71251 on the dorsal root- and saphenous nerve-evoked slow depolarizing responses were examined. Hemisected spinal cords were used in other experiments. The preparation was placed in a recording chamber of 0.5 ml volume and perfused with artificial cerebrospinal fluid (CSF) saturated with 95% O_2:5% CO_2 at a flow rate of 2–4 ml min^{-1}. The compositon of artificial CSF was as follows (in mM): NaCl 138.6, KCl 3.35, $CaCl_2$ 1.26, $MgCl_2$ 1.15, $NaHCO_3$ 21.0, NaH_2PO_4 0.58, glucose 10.0. In the experiments in which the effects of GR71251 on the action of exogenous tachykinins and other peptides were examined (Figures 1, 2, 3 and 4), the concentration of $MgCl_2$ was increased to 2 mM in order to depress spontaneous activity. The temperature of the perfusion medium was kept at 27°C. Drugs were dissolved in artificial CSF and applied to the spinal cord by perfusion.

Electrophysiology and data analysis

Suction electrodes were used for electrical stimulation of and extracellular recording from L3-L5 nerve roots. Changes in potential of the ventral root were led to a d.c. amplifier and then to a pen recorder and a computer recording device (Axotape). Spinal reflexes of fast time-course were stored in a memory device and then displayed on a pen-recorder with a slower time scale.

Effects of drugs on the contralateral dorsal root-evoked slow ventral root potentials (VRPs) were investigated in the whole spinal cord preparation (Akagi *et al.*, 1985). Stimulation with five shocks (40 V intensity, 300 μs duration at 20 Hz) were given to a dorsal root every 90 s and the resultant reflex responses were recorded extracellularly from the contralateral ventral root of the same segment. The average areas (mV·s) of 3 consecutive responses were measured before and after adding each antagonist. GR71251 was applied in a cumulative manner (5 and 10 μM), for 20 min for each concentration. After washing out GR71251 for at least 30 min, spantide was applied for 30 min.

Effects of drugs on the cutaneous nerve-evoked slow depolarizing responses were investigated in the spinal cord-saphenous preparation (Nussbaumer *et al.*, 1989). Stimulation with two shocks (40 V intensity, 100 μs duration at 20 Hz) were given to the saphenous nerve every 3 min and the resultant reflex responses were recorded extracellularly from the ipsilateral L3 ventral root. The average areas (mV·s) of 3 consecutive responses were measured before and after adding GR71251.

To investiage the cutaneous nerve-evoked inhibition of monosynaptic reflex in the spinal cord-peripheral nerve preparation (Yoshioka *et al.*, 1990), single-shock test stimuli (a square pulse of 40–50 V in amplitude and 200–500 μs in duration, i.e. of supramaximum intensity for monosynaptic reflex) were applied to a nerve branch of the quadriceps femoris muscle every 120 s. The resulting reflex responses were recorded from the L3 ventral root. Conditioning stimuli (2–5 shocks of 200–500 μs duration, at 20–50 Hz and 40–50 V in intensity) were delivered to the saphenous nerve. Conditioning-test intervals were altered in a decreasing order usually starting at 20 s, and the amplitude of the conditioned monosynaptic reflex was expressed as a percentage of the control reflex amplitude (the amplitudes of monosynaptic reflexes immediately before the conditioning stimulation).

To examine the effects of GR71251 and spantide on the capsaicin-induced depolarization, capsaicin was bath-applied for 30 s every 30 min to the spinal cord preparation. GR71251 was applied at two concentrations (5 and 10 μM) for 10 min. After washing out GR71251 for at least 60 min, spantide (15 μM) was applied for 15 min.

Estimation of pA_2 values

Tachykinin agonists were bath-applied for 30 s at 10 to 30 min intervals to the spinal cord preparation and the area of the depolarization (mV·s) was calculated. The concentration-response curves were constructed in normal artificial CSF and then after equilibration of the preparation for 8 min with GR71251 or for 15 min with spantide at two different concentrations. The antagonist-induced displacement of agonist concentration-response curves was quantified as the ratio of equi-active molar concentrations at the half-maximum response level of the control concentration-response curve and the pA_2 value was determined from Arunlakshana-Schild plots (Arunlakshana & Schild, 1959).

Drugs

GR71251 ([D-Pro^9[spiro-γ-lactam]Leu^{10},Trp^{11}]SP) was synthesized as previously described (Ward *et al.*, 1990; Hagan *et al.*, 1990). Spantide ([D-Arg^1,D-Trp^{7,9},Leu^{11}]SP) was kindly supplied by Dr M. Fujino, Takeda Chemical Industries, Ltd. Japan. Acetyl-Arg^6-septide (acetyl-[Arg^6,Pro^9]SP_{6-11}, a water soluble form of septide with similar properties; Papir-Kricheli *et al.*, 1987) and senktide (succinyl-[Asp^6-MePhe^8]SP_{6-11}; Papir-Kricheli *et al.*, 1987) were gifts from Professor Z. Selinger, Department of Biological Chemistry, the Hebrew University of Jerusalem, Israel. SP, NKA, bombesin, gastrin-releasing peptide (GRP), neuromedin B and thyrotropin-releasing hormone (TRH) were purchased from Peptide Institute, Inc. Osaka, Japan. Neurokinin B (NKB), β-Ala^8NKA_{4-10} and Nle^{10}NKA_{4-10} were from Peninsula's Laboratory. Other drugs were obtained from various commercial sources.

Results

Effects of GR71251 and spantide on depolarization induced by tachykinin agonists in normal artificial CSF

Bath-application of SP, NKA and NKB at a concentration range of 0.01–3 μM to the spinal cord of the neonatal rat produced depolarization of ventral roots in normal artificial CSF. Acetyl-Arg^6-septide, a selective NK_1 agonist, and senktide, a selective NK_3 agonist, had also potent depolarizing effects, whereas β-Ala^8NKA_{4-10} and Nle^{10}NKA_{4-10}, which act as selective NK_2 agonists in peripheral tissues (Rovero *et al.*, 1989; Regoli *et al.*, 1990), had only minor depolarizing effects at 1 μM (Table 1).

Figure 1 illustrates the effect of GR71251 on the SP-induced depolarization, as compared with that of spantide, in the same preparation. Both GR71251 and spantide depressed the SP-induced response. The depressant effects of GR71251 at 3 μM and 5 μM were approximately the same as those of spantide at 10 μM and 15 μM, respectively (see Table 2). After removal of GR71251 the SP-induced response rapidly

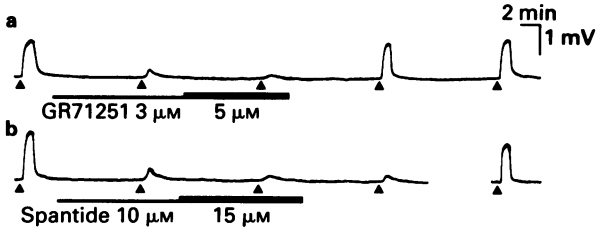

Figure 1 Comparison of the effects of GR71251 and spantide on the depolarization of the ventral root induced by substance P (SP). Extracellular recording was made from the L4 ventral root of a hemisected spinal cord preparation of 1 day-old rat. SP (0.1 μM) was bath-applied for 30 s at (▲) every 12 min. The effect of spantide was examined after complete recovery from the effect of GR71251 in the same preparation. (a) GR71251 at 3 and 5 μM was applied during the period shown by the thin and thick horizontal bars, respectively. (b) Spantide at 10 and 15 μM was applied during the period shown by the thin and thick horizontal bars, respectively. The trace at the right was recorded 120 min after removal of spantide.

Table 1 The potencies of tachykinin agonists in evoking ventral root depolarization

Agonist	*Area* (mV·s)	n
Substance P	203.5 ± 16.4	18
Neurokinin A	187.6 ± 19.4	9
Neurokinin B	253.3 ± 10.6	4
Acetyl-Arg^6-septide	529.5 ± 78.9	7
Senktide	355.8 ± 41.0	5
β-Ala^{8}NKA$_{4-10}$	3.8 ± 1.4	5
Nle^{10}NKA$_{4-10}$	3.1 ± 1.2	5

All agonists were bath-applied at 1 μM for 30 s. The areas of depolarization of the ventral root were measured and expressed as mean ± s.e.mean.

Table 2 pA_2 values and slopes of the Schild plot for GR71251 against tachykinin agonist-evoked depolarization in normal artificial CSF

Antagonist and Agonist	*pA_2*	*Slope*	n
GR71251			
Substance P	6.14 ± 0.03	0.97 ± 0.02	6
Neurokinin A	6.75 ± 0.03*	1.00 ± 0.01	4
Acetyl-Arg^6-septide	6.70 ± 0.08*	0.99 ± 0.03	5
Spantide			
Substance P	5.88 ± 0.03*	0.77 ± 0.04	6
Bombesin	5.29 ± 0.02	1.12 ± 0.09	4

The pA_2 values were determined from Aunlakshana-Schild plots. Data are expressed as mean ± s.e.mean.
*Significantly different from the value of GR71251 against substance P by unpaired t test at $P<0.001$.

returned to its original size in 10–20 min, whereas the recovery from the action of spantide occurred much more slowly and often incompletely within 1–2 h (Figure 1b). GR71251 or spantide alone did not alter the potential of the ventral root at a concentration range of 0.3–30 μM.

In the experiments shown in Figure 2 and Table 2, the effects of GR71251 on the depolarizing responses of the ventral root to SP, acetyl-Arg^6-septide, NKA, senktide and NKB were examined. GR71251 (0.3–3 μM) caused a rightward shift of the concentration-response curves for SP, acetyl-Arg^6-septide and NKA (Figures 2a,b and c). About 20 min after removal of GR71251, the curves for these agonists were similar to controls. The slope of the Schild plot for GR71251 against SP, acetyl-Arg^6-septide and NKA was close to unity (Table 2). The estimated pA_2 values for GR71251 against NKA, acetyl-Arg^6-septide were about the same (6.7–6.8) whereas the pA_2 against SP was slightly lower (6.14). In contrast, the depolarizing responses to NKB and senktide were little affected by GR71251 at 1–5 μM (Figure 2d,e). Thus, in the presence of GR71251 (3–5 μM), the depolarizing responses to NKB (0.3 μM) and senktide (0.3 μM) were 86.6 ± 10.4% ($n = 3$) and 78.3 ± 14.5% ($n = 3$) of the control responses (statistically not significant at $P<0.05$, by t test), respectively. The effects of GR71251 on β-Ala^{8}NKA$_{4-10}$ and Nle^{10}NKA$_{4-10}$ actions were not examined in the present study because of their minor effects in this preparation.

Specificity of GR71251

Figure 3 shows the effects of GR71251 on the responses to bombesin, and two mammalian bombesin-like peptides, GRP and neuromedin B. When these agonists were bath-applied for 30 s in normal artificial CSF, depolarizing responses were induced in ventral roots. GR71251 (1–3 μM) caused no shift of the concentration-response curves for these agonists. The response to bombesin at 0.01–0.03 μM in the presence of

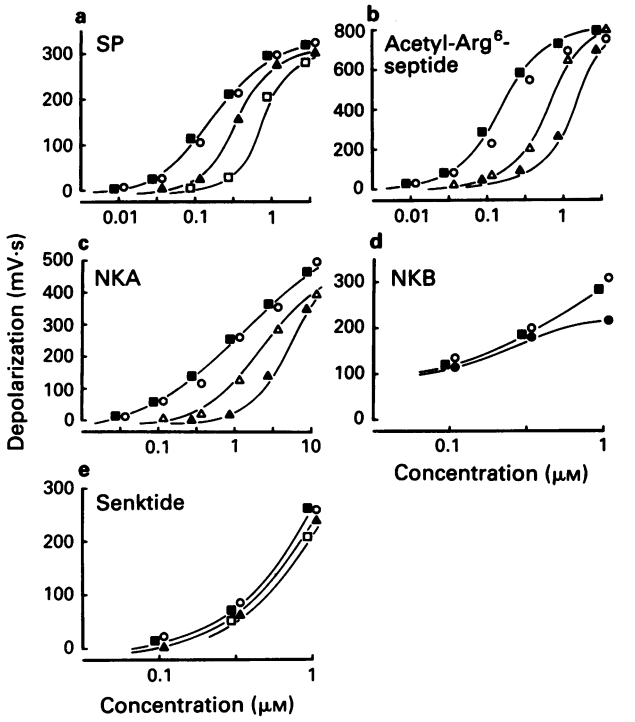

Figure 2 Effects of GR71251 on the concentration-response curves for substance P (SP), acetyl-Arg^6-septide, neurokinin A (NKA), senktide and neurokinin B (NKB) in normal artificial CSF. The tachykinin receptor agonists were applied by perfusion for 30 s. The area of depolarization of the ventral root is plotted against logarithmic concentrations of the agonists. The positions of symbols are horizontally adjusted to avoid their overlaps. (■) In normal artificial CSF; (△), (▲), (□) and (●) after addition of GR71251 at 0.3 μM, 1 μM, 3 μM and 5 μM, respectively; (○) 20–80 min (a,b,c) and 120–200 min (d,e) after removal of GR71251.

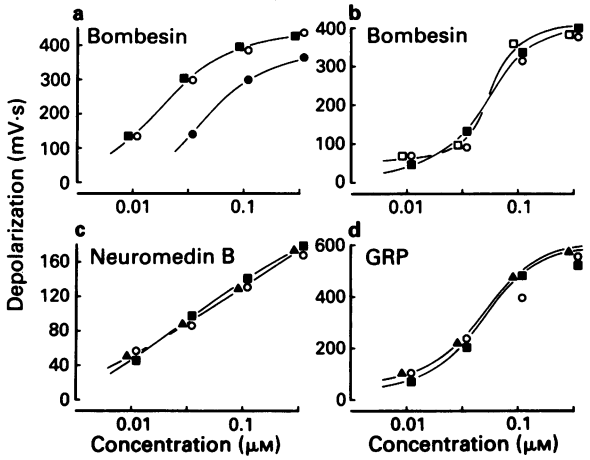

Figure 3 Effects of spantide (a) and GR71251 (b,c,d) on the concentration-response curves for bombesin, and bombesin-like peptides, neuromedin B and gastrin releasing peptide (GRP). These agonists were applied by perfusion for 30 s. The area of depolarization of the ventral root is plotted against logarithmic concentrations of the agonists. The positions of symbols are horizontally adjusted to avoid their overlaps: (a) (■) in normal artificial CSF; (●) after addition of spantide 15 μM; (○) 60–240 min after removal of spantide. (b,c,d) (■) In normal artificial CSF; (▲) and (□) after addition of GR71251 at 1 μM and 3 μM, respectively; (○) 60–240 min after removal of GR71251.

GR71251 at 3–5 μM was 103.2 ± 7.6% of the control responses ($n = 5$), whereas spantide at 15 μM caused a rightward shift of the concentration-response curve for bombesin (Figure 3a). The estimated pA_2 value for spantide against bombesin was 5.29 ± 0.02 ($n = 4$) (Table 1). After removal of

spantide the responses to bombesin returned to original sizes in 60–240 min.

The effects of GR71251 on the responses to other agonists, such as L-glutamate, acetylcholine, TRH, GABA and noradrenaline were examined in the presence of tetrodotoxin (TTX) at 0.3 μM. When these agonists were bath-applied for 30 s, they produced depolarizing responses. GR71251 at 1 μM did not affect the depolarization induced by L-glutamate (0.03–3 mM), acetylcholine (0.03–3 mM), GABA (0.03–3 mM) and noradrenaline (1–30 μM) (not shown). The depolarizing action of TRH was potentiated by GR71251 (1 μM). Thus, in the presence of GR71251 (1 μM), the depolarizing response to TRH at 0.3 μM was potentiated by 28.7 ± 2% ($n = 3$) and at 1 μM, by 13 ± 1% ($n = 3$), respectively, statistically significant at $P < 0.001$, by t test.

Effects of GR71251 on the capsaicin-induced depolarization

Bath-application of 0.3 μM-capsaicin for 20 s produced a depolarizing response similar to the response to SP at 20 nM applied for 30 s (Figure 4). While the SP-induced depolarization was almost completely depressed by GR71251 at 3–10 μM, the capsaicin-induced response was only partially depressed by GR71251: i.e. the initial part of the response was resistant to GR71251 at 3–10 μM, whereas the later part was completely depressed by GR71251 at 3 μM ($n = 4$). Spantide also exerted similar effects. However, the maximum depressant effect of GR71251, which was observed at 5–10 μM, was slightly but significantly smaller than that of spantide at 15 μM (Table 3).

Effects of GR71251 on the dorsal root-evoked spinal reflexes

Intense electrical stimulation of a dorsal root evoked in the contralateral ventral root of the corresponding segment a slow depolarizing response lasting about 20 s (Figure 5). This reflex response which is referred to as contralateral slow ventral root potential (VRP), has been shown to involve activation of primary afferent C-fibres and to be depressed by tachykinin antagonists, [D-Arg^1,D-Pro^2,D-Trp^{7,9},Leu^{11}]-SP (Yanagisawa *et al.*, 1982; Akagi *et al.*, 1985) and spantide (Otsuka & Yanagisawa, 1988). GR71251 (3–10 μM) markedly depressed the contralateral slow VRP and recovery occurred in 10–20 min after removal of the antagonist ($n = 4$) (Figure 5b). The depressant effect of GR71251 became maximum at 5 μM (Table 3). Spantide at 15 μM, which was approximately equipotent to GR71251 at 5 μM in antagonizing the depolarizing action of SP, showed a slightly but significantly more pronounced depressant effect than GR71251 at 5 μM on the contralateral slow VRP (Table 2). Recovery of the response after removal of spantide was much slower taking 60–90 min.

Single-shock stimulation of a dorsal root evoked in the ipsilateral ventral root of the same segment monosynaptic and polysynaptic reflexes of fast time course which were followed by a slow depolarization (ipsilateral slow VRP; Otsuka & Yanagisawa, 1988). GR71251 (1–3 μM) exerted a depressant effect on the ipsilateral slow VRP but did not affect the monosynaptic and polysynaptic reflexes of fast time course (not shown). The latter finding suggests that GR71251 at 3 μM has no local anaesthetic action.

Effects of GR71251 on the saphenous nerve-evoked depolarization

Our previous studies using isolated spinal cord-saphenous nerve preparation showed that electrical stimulation of the saphenous nerve at strength sufficient to activate C fibres evoked a slow depolarizing potential of approximately 20 s duration in the L3 ventral root and this potential was depressed by spantide (Nussbaumer *et al.*, 1989). GR71251

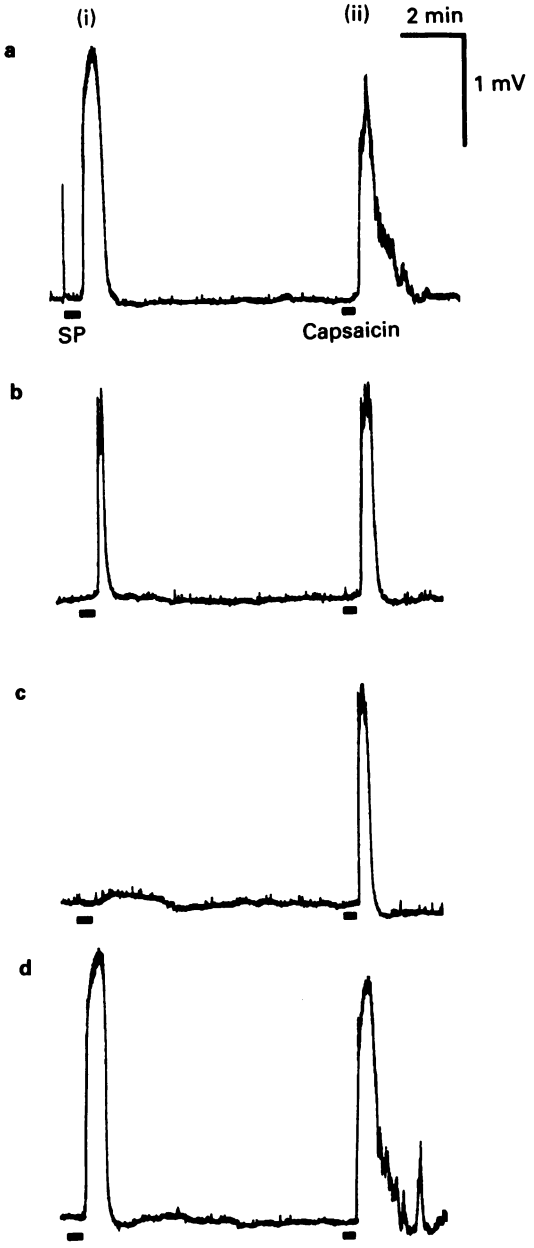

Figure 4 Effects of GR71251 on the responses to substance P (SP) and capsaicin. Responses were recorded from the L4 ventral root of a hemisected spinal cord preparation of 1 day-old rat. SP (10 nM) (i) and capsaicin (0.3 μM) (ii) were bath-applied for 30 s and 20 s, respectively, at 10 min intervals; (a) control responses; (b) and (c) 8–18 min after addition of GR71251 at 3 μM and 10 μM, respectively; (d) 40–50 min after removal of GR71251.

(0.3–3 μM) likewise depressed the saphenous nerve-evoked slow depolarization. This recovered rapidly after the removal of GR71251 ($n = 5$) (Figure 6).

Effects of GR71251 on the saphenous nerve-evoked inhibition of monosynaptic reflex

In the spinal cord-peripheral nerve preparation, conditioning electrical stimulation of the saphenous nerve inhibited (for about 20 s) the monosynaptic reflex elicited by stimulation of quadriceps femoris nerve. In the experiment illustrated in Figure 7, test monosynaptic reflexes were elicited by stimulations of the quadriceps femoris nerve and recorded from the L3 ventral root of an isolated spinal cord-peripheral nerve preparation. Conditioning stimulation with 2–5 shocks

Table 3 Effects of GR71251 and spantide on the capsaicin-induced depolarization and contralateral slow VRP

	GR71251 (5 μM)	*GR71251* (10 μM)	*Spantide* (15 μM)	n
Capsaicin-induced response	56.7 ± 5.1	57.7 ± 1.6	34.1 ± 5.9*†	4
Contralateral slow VRP	61.4 ± 3.0	60.8 ± 3.8	50.7 ± 4.0*	3

For the capsaicin-induced response the areas of depolarization induced by capsaicin (0.1–0.5 μM, 30 s) were measured before and after adding each antagonist. For the contralateral slow VRP evoked by the dorsal root the average areas of 3 consecutive responses were measured before and after adding each antagonist. Each value represents % of control response and is expressed as mean ± s.e.mean.

*Significantly different from the value for GR71251 (5 μM) by paired t test at $P<0.05$; †Significantly different from the value for GR71251 (10 μM) by paired t test at $P<0.05$. The values for GR71251 at 5 μM and 10 μM in inhibiting both the capsaicin-induced response and the contralateral slow VRP were not significantly different from each other at $P<0.05$ by paired t test.

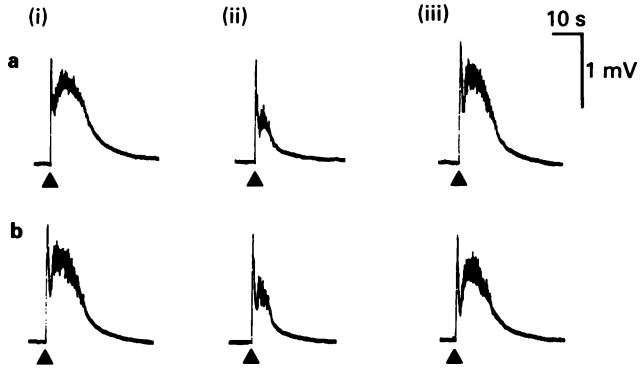

Figure 5 Effects of GR71251 and spantide on the contralateral slow ventral root potential (VRP). Records from an isolated spinal cord preparation of 2 day-old rat. Stimulation with five shocks (40 V intensity, 300 μs duration at 20 Hz) were given at (▲) to an L4 dorsal root every 90 s and the resultant reflex responses were recorded extracellularly from the L4 ventral root of the contralateral side. (i) Control response; (ii) 12–14 min after addition of spantide at 15 μM (a) and GR71251 at 10 μM (b); (iii) 120 and 30 min after removal of spantide (a) and GR71251 (b), respectively.

(40–50 V intensity and 200–500 μs duration at 50 Hz) was applied to the saphenous nerve every 120 s (Yoshioka *et al.*, 1990). GR71251 (1–3 μM) markedly reduced the saphenous nerve-evoked inhibition of the monosynaptic reflex, particularly at conditioning-test intervals of 5–20 s. This recovered rapidly after the removal of GR71251 ($n=6$).

Discussion

GR71251 had little depressant action on the depolarizing response to bombesin, GRP or neuromedin B in the neonatal rat spinal cord whereas spantide markedly depressed the response to bombesin (Figure 3; Otsuka & Yanagisawa, 1988; Jensen & Coy, 1991; Rouissi *et al.*, 1991). GR71251 had also virtually no antagonist action on the depolarization induced by NKB and senktide. Furthermore, GR71251 did not alter the concentration-response curves for L-glutamate, acetylcholine, TRH, GABA and noradrenaline. Therefore, the present study shows that GR71251 is more selective than

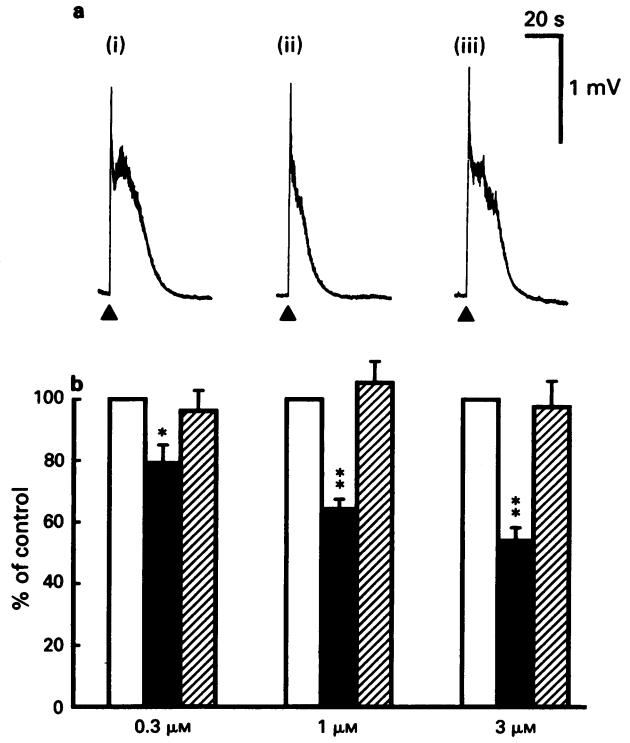

Figure 6 Effect of GR71251 on the saphenous nerve-evoked slow VRP. (a) Experiment in an isolated spinal cord-peripheral nerve preparation of a 2 day-old rat. The saphenous nerve was stimulated every 3 min at (▲) with 2 shocks (40 V intensity and 100 μs duration at 20 Hz) and the potential was recorded from the ipsilateral L3 ventral root. (i) Control response; (ii) after addition of GR71251 at 3 μM; (iii) after removal of GR71251. (b) Dose-dependently of the effect of GR71251 on the saphenous nerve-evoked slow VRP. The areas of the responses in mV·s were measured and shown as percentages of the average of control responses. Open columns represent control responses; solid columns, after addition of GR71251 at 0.3, 1 and 3 μM respectively; hatched columns, after removal of GR71251. Each column and vertical bar express mean ± s.e.mean ($n=5$). Significantly different from the control values at $*P<0.05$ and $**P<0.001$ by paired t test.

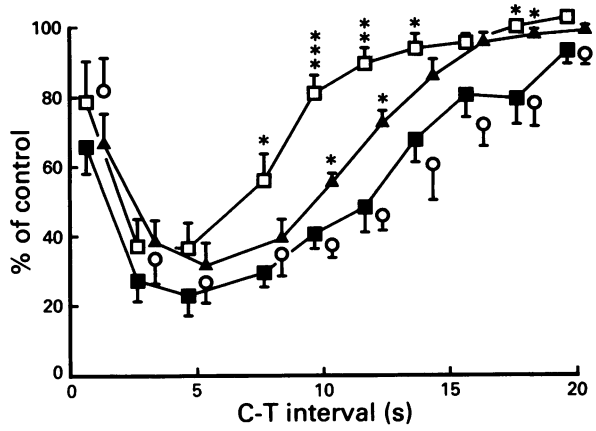

Figure 7 Effects of GR71251 on the saphenous nerve-evoked inhibition of monosynaptic reflex. Test monosynaptic reflexes were elicited by stimulations of the quadriceps femoris nerve and recorded from the L3 ventral root of an isolated spinal cord-peripheral nerve preparation. Conditioning stimulation with 2–5 shocks (40–50 V intensity and 200–500 μs duration at 50 Hz) was applied to the saphenous nerve every 120 s. The positions of symbols are horizontally adjusted to avoid overlapping. (■) Inhibition in normal artificial CSF; (▲) and (□) after addition of GR71251 at 1 μM and 3 μM, respectively; (○) 60 min after removal of GR71251. Each point expresses mean ± s.e.mean ($n=6$). Significantly different from the corresponding control values at $*P<0.05$; $**P<0.01$, and $***P<0.001$ by unpaired t test.

spantide as a tachykinin antagonist in the neonatal rat spinal cord. In addition, GR71251 is superior to spantide as an experimental tool in that it is more potent and its effect is readily reversed after removal.

Previous studies showed that both the dorsal root- or saphenous nerve-evoked slow VRP and the saphenous nerve-evoked inhibition of the monosynaptic reflex were depressed by the tachykinin antagonists, [D-Arg[1],D-Pro[2],D-Trp[7,9],Leu[11]]-SP and/or spantide (Yanagisawa *et al.*, 1982; Akagi *et al.*, 1985; Otsuka & Yanagisawa, 1988; Nussbaumer *et al.*, 1989; Yoshioka *et al.*, 1990). The depolarization of ventral roots induced by application of capsaicin to the spinal cord was also depressed by spantide (Yoshioka *et al.*, 1990). These results suggested that tachykinins are involved in these responses. However, because of the blocking action of these antagonists on bombesin receptors (Folkers *et al.*, 1984; Otsuka & Yanagisawa, 1988; Jensen *et al.*, 1988), the possibility remained that bombesin-like peptides, but not tachykinins, contribute to these responses. Indeed, neuromedin B mRNA and GRP mRNA have been found in rat dorsal root ganglia and the spinal cord, respectively (Wada *et al.*, 1990). Bombesin-like immunoreactivity has also been demonstrated in a subpopulation of mammalian primary sensory neurones (Fuxe *et al.*, 1983; Panula *et al.*, 1983; Cameron *et al.*, 1988) and certain spinal neurones (Leah *et al.*, 1988), although the amounts of the immunoreactivity were much smaller than those of SP in the spinal cord and sensory ganglia (McGregor *et al.*, 1984; Yaksh *et al.*, 1988). In the present study, GR71251 depressed both the dorsal root- and saphenous nerve-evoked slow VRPs and the saphenous nerve-evoked inhibition of monosynaptic reflex. GR71251 also depressed the later slow component of the capsaicin-induced depolarization. The present study, therefore, adds further support for the involvement of SP and NKA in these responses. Spantide exerted a slightly greater depressant effect than GR71251 on the dorsal root-evoked contralateral slow VRP as well as the capsaicin-induced depolarization (Table 3). Whether this is due to the contribution to these responses of other peptides, such as NKB and bombesin-like peptides, remains to be clarified. The partial blockade of the capsaicin-evoked depolarization by GR71251 and spantide (Table 3) suggests that it is due to a release of both tachykinins and other sensory transmitters (cf. Urbán & Dray, 1992).

The pA_2 of GR71251 against the depolarizing action of NKA was close to the pA_2 against acetyl-Arg[6]-septide. This suggests that GR71251, NKA and acetyl-Arg[6]-septide bind to a single class of tachykinin receptors. The pA_2 value of GR71251 against NKA obtained in this study (6.75) was much higher than the pA_2 of GR71251 against the NK_2 receptor-mediated contractile action of NKA in the rat colon muscularis mucosae (4.8) (Ward *et al.*, 1990). Furthermore, β-Ala[8]NKA_{4-10} and Nle[10]NKA_{4-10}, two selective NK_2 agonists that selectively activate at NK_2 receptors in peripheral tissues (Rovero *et al.*, 1989; Regoli *et al.*, 1990), showed little depolarizing effect at 1 μM. Since it was known in peripheral tissues that acetyl-Arg[6]-septide and NKA preferentially act on NK_1 and NK_2 receptors, respectively, the receptor to which NKA and acetyl-Arg[6]-septide bind as observed in the present study appears to be distinct from NK_1, NK_2 or NK_3 receptor found in peripheral tissues. The existence of this novel type of receptor was suggested previously based on the similar antagonist profile of spantide against the depolarizing responses of motoneurones to NKA and acetyl-Arg[6]-septide (Yanagisawa & Otsuka, 1990). On the other hand, the significantly lower pA_2 of GR71251 against the SP-induced depolarizing responses than against the NKA- and acetyl-Arg[6]-septide-induced responses may reflect involvement of another type of tachykinin receptor in the SP-induced response. Furthermore, there was a slight tendency for the concentration-response curves to become steeper in the presence of increasing concentrations of GR71251 (Figure 2). This may indicate that the responses to these agonists involve more than one type of receptor. These possibilities need to be examined further by the use of other selective tachykinin agonists and antagonists.

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References

AKAGI, H., KONISHI, S., OTSUKA, M. & YANAGISAWA, M. (1985). The role of substance P as a neurotransmitter in the reflexes of slow time courses in the neonatal rat spinal cord. *Br. J. Pharmacol.*, **84**, 663–673.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.

CAMERON, A.A., LEAH, J.D. & SNOW, P.J. (1988). The coexistence of neuropeptides in feline sensory neurons. *Neuroscience*, **27**, 969–979.

FOLKERS, K., HÅKANSON, R., HÖRIG, J., XU, J.-C. & LEANDER, S. (1984). Biological evaluation of substance P antagonists. *Br. J. Pharmacol.*, **83**, 449–456.

FUXE, K., AGNATI, L.F., MCDONALD, T., LOCATELLI, V., HÖKFELT, T., DALSGAARD, C.-J., BATTISTINI, N., YANAIHARA, N., MUTT, V. & CUELLO, A.C. (1983). Immunohistochemical indications of gastrin releasing peptide – bombesin-like immunoreactivity in the nervous system of the rat. Codistribution with substance P-like immunoreactive nerve terminal systems and coexistence with substance P-like immunoreactivity in dorsal root ganglion cell bodies. *Neurosci. Lett.*, **37**, 17–22.

GUO, J.-Z., YOSHIOKA, K., YANAGISAWA, M., HAGAN, R.M. & OTSUKA, M. (1992). Pharmacological profile of GR71251, a new tachykinin receptor antagonist, in the isolated spinal cord of the newborn rat (abstract). *Jpn. J. Pharmacol.*, **58** (Suppl.), 262P.

GUO, J.-Z., YOSHIOKA, K., YANAGISAWA, M., HAGAN, R.M. & OTSUKA, M. (1993). Blockade of cutaneous nerve-evoked responses by GR71251 in the isolated spinal cord preparation of newborn rat. *Regul. Pept.*, **46**, 309–310.

HAGAN, R.M., IRELAND, S.J., JORDAN, C.C., BERESFORD, I.J.M., STEPHENS-SMITH, M.L., EWAN, G. & WARD, P. (1990). GR71251, a novel, potent and highly selective antagonist at neurokinin NK-1 receptors (abstract). *Br. J. Pharmacol.*, **99**, 62P.

HALL, J.M. & MORTON, I.K.M. (1991). Novel selective agonists and antagonists confirm neurokinin NK_1 receptors in guinea-pig vas deferens. *Br. J. Pharmacol.*, **102**, 511–517.

IRELAND, S.J., BAILEY, F., COOK, A., HAGAN, R.M., JORDAN, C.C. & STEPHENS-SMITH, M.L. (1991). Receptors mediating tachykinin-induced contractile responses in guinea-pig trachea. *Br. J. Pharmacol.*, **103**, 1463–1469.

JENSEN, R.T. & COY, D.H. (1991). Progress in the development of potent bombesin receptor antagonists. *Trends Pharmacol. Sci.*, **12**, 13–19.

JENSEN, R.T., HEINZ-ERIAN, P., MANTEY, S., JONES, S.W. & GARDNER, J.D. (1988). Characterisation of ability of various substance P antagonists to inhibit action of bombesin. *Am. J. Physiol.*, **254**, G883–G890.

JENSEN, R.T., JONES, S.W., FOLKERS, K. & GARDNER, J.D. (1984). A synthetic peptide that is a bombesin receptor antagonist. *Nature*, **309**, 61–63.

LEAH, J., MENÉTREY, D. & DE POMMERY, J. (1988). Neuropeptides in long ascending spinal tract cells in the rat: evidence for parallel processing of ascending information. *Neuroscience*, **24**, 195–207.

McGREGOR, G.P., GIBSON, S.J., SABATE, I.M., BLANK, M.A., CHRISTOFIDES, N.D., WALL, P.D. & POLAK, J.M. (1984). Effect of peripheral nerve section and nerve crush on spinal cord neuropeptides in the rat; increased VIP and PHI in the dorsal horn. *Neuroscience*, **13**, 207–216.

MIZRAHI, J., DION, S., D'ORLÉANS-JUSTE, P. & REGOLI, D. (1985). Activities and antagonism of bombesin on urinary smooth muscles. *Eur. J. Pharmacol.*, **111**, 339–345.

NUSSBAUMER, J.-C., YANAGISAWA, M. & OTSUKA, M. (1989). Pharmacological properties of a C-fibre response evoked by saphenous nerve stimulation in an isolated spinal cord-nerve preparation of the newborn rat. *Br. J. Pharmacol.*, **98**, 373–382.

OTSUKA, M. & YANAGISAWA, M. (1988). Effect of a tachykinin antagonist on a nociceptive reflex in the isolated spinal cord-tail preparation of the newborn rat. *J. Physiol.*, **395**, 255–270.

PANULA, P., HADJICONSTANTINOU, M., YANG, H.-Y.T. & COSTA, E. (1983). Immunohistochemical localization of bombesin/gastrin-releasing peptide and substance P in primary sensory neurons. *J. Neurosci.*, **3**, 2021–2029.

PAPIR-KRICHELI, D., FREY, J., LAUFER, R., GILON, C., CHOREV, M., SELINGER, Z. & DEVOR, M. (1987). Behavioural effects of receptor-specific substance P. *Pain*, **31**, 263–276.

POST, C., BUTTERWORTH, J.F., STRICHARTZ, G.R., KARLSSON, J.-A. & PERSSON, C.G.A. (1985). Tachykinin antagonists have potent local anaesthetic actions. *Eur. J. Pharmacol.*, **117**, 347–354.

REGOLI, D., RHALEB, N.-E., DION, S., TOUSIGNANT, C., ROUISSI, N., JUKIC, D. & DRAPEAU, G. (1990). Neurokinin A. A pharmacological study. *Pharmacol. Res.*, **22**, 1–14.

ROUISSI, N., RHALEB, N.-E., NANTEL, F., DION, S., DRAPEAU, G. & REGOLI, D. (1991). Characterization of bombesin receptors in peripheral contractile organs. *Br. J. Pharmacol.*, **103**, 1141–1147.

ROVERO, P., PESTELLINI, V., PATACCHINI, R., GIULIANI, S., SANTICIOLI, P., MAGGI, C.A., MELI, A. & GIACHETTI, A. (1989). A potent and selective agonist for NK-2 tachykinin receptor. *Peptides*, **10**, 593–595.

URBÁN, L. & DRAY, A. (1992). Synaptic activation of dorsal horn neurons by selective C-fiber excitation with capsaicin in the mouse spinal cord *in vitro*. *Neuroscience*, **47**, 693–702.

WADA, E.D, WAY, J., LEBACQ-VERHEYDEN, A.M. & BATTEY, J.F. (1990). Neuromedin B and gastrin-releasing peptide mRNAs are differentially distributed in the rat nervous system. *J. Neurosci.*, **10**, 2917–2930.

WARD, P., EWAN, G.B., JORDAN, C.C., IRELAND, S.J., HAGAN, R.M. & BROWN, J.R. (1990). Potent and highly selective neurokinin antagonists. *J. Med. Chem.*, **33**, 1848–1851.

YACHNIS, A.T., CRAWLEY, J.N., JENSEN, R.T., McGRANE, M.M. & MOODY, T.W. (1984). The antagonism of bombesin in the CNS by substance P analogues. *Life Sci.*, **35**, 1963–1969.

YAKSH, T.L., MICHENER, S.R., BAILEY, J.E., HARTY, G.J., LUCAS, D.L., NELSON, D.K., RODDY, D.R. & GO, V.L. (1988). Survey of distribution of substance P, vasoactive polypeptide, cholecystokinin, neurotensin, Met-enkephalin, bombesin and PHI in the spinal cord of cat, dog, sloth and monkey. *Peptides*, **9**, 357–372.

YANAGISAWA, M. & OTSUKA, M. (1990). Pharmacological profile of a tachykinin antagonist, spantide, as examined on rat spinal motoneurones. *Br. J. Pharmacol.*, **100**, 711–716.

YANAGISAWA, M., OTSUKA, M., KONISHI, S., AKAGI, H., FOLKERS, K. & ROSELL, S. (1982). A substance P antagonist inhibits a slow reflex response in the spinal cord of the newborn rat. *Acta Physiol. Scand.*, **116**, 109–112.

YOSHIOKA, K., SAKUMA, M. & OTSUKA, M. (1990). Cutaneous nerve-evoked cholinergic inhibition of monosynaptic reflex in the neonatal rat spinal cord: involvement of M_2 receptors and tachykininergic primary afferents. *Neuroscience*, **38**, 195–203.

YOSHIZAWA, T., KANAZAWA, I. & NAKANISHI, T. (1987). Substance P antagonist (spantide) suppresses the compound action potentials of the rat sciatic nerve in vitro. *Neuropeptide*, **10**, 181–188.

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